Self-Folding Hydrogel Bilayer for Enhanced Drug Loading, Encapsulation, and Transport

Hen-Wei Huang[1], Andrew J. Petruska[1], Mahmut Selman Sakar[2], Maria Skoura[1], Franziska Ullrich[1], Qi Zhang[1,3], Salvador Pané[1], and Bradley J. Nelson[1]

***Abstract*— Hydrogel-based robotic microdevices are currently investigated for minimally invasive medical procedures. Hydrogels are especially suited to targeted drug delivery applications as they are able to carry several times more drug solution than its dry weight. A major drawback of these system is that drug release takes place before reaching the targeted area in the body. We introduce a strategy based on a self-folding bilayer to prevent release during transportation without hindering the drug loading efficiency of the hydrogel. The drug is loaded into the hydrogel matrix at room temperature. When the temperature is increased to body temperature, the hydrogel-matrix collapses and the self-folded bilayer refolds into another tube. In this configuration, we observed a significant reduction in drug leakage with less than 5% drug loss during encapsulation. Finally, we demonstrate that the tube can be manipulated magnetically, which shows its potential use in targeted drug delivery applications.**

I. Introduction

Targeted therapies enable a clinician to directly control the concentration of medicine in a target region [1]. This therapy is largely founded on hydrogel-based robotic microdevices (HRMD) [2, 3]. Hydrogels are employed in encapsulation and delivery of drugs [4, 5] because it exhibits properties such as high-swelling, biocompatibility, biodegradability, and stimuli response [6]. The high-swelling property endows HRMDs not only with exceptional drug loading ability [7], but also with self-folding capability through stress-induced bending [8]. Thus, HRMDs are able to form 3D structures from 2D patterns when the drug solution is loaded into the hydrogel. Moreover, HRMDs are able to control the rate of drug release [9].

Using hydrogels for drug delivery is advantageous but the high water content of hydrogels results in the rapid release of drugs from the gel matrix in a couple of hours to days. The rate of release can be tuned by adjusting the cross-linking degree in the gel to control pore size. However, a highly cross-linked gel exhibits reduced swelling and slow response to environmental stimuli [4]. To date, existing HRMDs suffer from either excessive drug leakage during the transportation to targeted areas or low efficiency in drug loading [10]. A sustainable release can be engineered by simultaneously UV-polymerizing the pre-gel polymer and drug molecules, as this process traps the molecules in a densely crosslinked polymer network [11, 12]. However, this process is only compatible with drugs that are insensitive to UV-light.

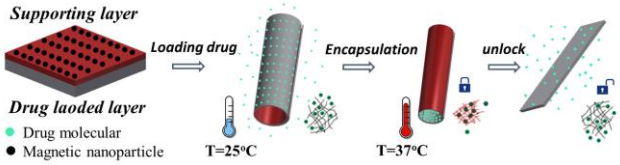

Figure 1. Programmable hydrogel bilayer for drug loading, encapsulation and release. The drug is loaded at room temperature. Once the temperature is increased to body temperature, the tubes refold and encapsulate the drug, protecting it from the environment. The drug release is enhanced when the supporting layer degrades inside the body once it has reached its target location.

Moreover, non-polymerized pre-gel solutions that are potentially toxic may diffuse out with the drug.

In this work, we propose a HRMD with a reconfigurable structure based on a self-folding hydrogel bilayer. The bilayer is composed of a thermally-responsive hydrogel with a relatively high-swelling property as the drug-loaded layer and a degradable magnetic nanocomposite [10] as a supporting layer. The drug-loaded layer carries the drug and controls the temperature dependent folding simultaneously. The magnetic nanocomposite both controls the folded shape and enables the tube to be manipulated magnetically. After the drug is completely loaded in the hydrogel matrix, the drug is encapsulated by increasing the temperature to body temperature (37 ˚C). This results in collapsing the hydrogel matrix, which reduces drug diffusion [13] and triggers the tube to refold such that the drug loaded layer is shielded from the external environment (Fig. 1). Then, the tube can be guided by a magnetic manipulation system to reach target regions without releasing the drug along the way. Finally, the release of the loaded drug is enhanced when the supporting layer degrades and the tube unfolds.

II. Materials and Methods

A. Material preparation for self-folding bilayers

A plastic photomask foil is designed and printed (Selba SA, Switzerland). The designed features are transferred on a glass wafer by photolithography, using photoresist S1813. Following that, a 100 nm thick layer of Chromium (Cr) is deposited on the glass wafer and a lift-off process is performed to obtain the final glass photomask. Spacers with a defined thickness are produced by photolithography by using SU-8 photoresist on silicon substrates. These spacer wafers are coated with Silane (Sigma Aldrich) by evaporation in a vacuum chamber to achieve a nonadhesive hydrophobic condition.

[1] Institute of Robotics and Intelligent Systems, ETH Zurich, 8092, Zurich, Switzerland. Email: hhuang@ethz.ch
[2] Institute of Mechanical Engineering, EPFL, 1015, Lausanne, Switzerland.
[3] Shenzhen Institutes of Advance Technology, Chinese Academy of Sciences.

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A thermally-responsive hydrogel is incorporated as a relatively high-swelling layer into the self-folding-bilayer structures. N-Isopropylacrylamide (NIPAAm) is used as the thermally-responsive monomer, while polyethylenglycol diacrylate (PEGDA) is used as the cross-linker. The molar ratio between NIPAAm and PEGDA is 100:1. The photo-initiator 2, 2 dimethoxy 2 phenylacetophenone (99%, EMPA), and the solvent ethyl lactate are added at a quantity of 3 wt% and 70 wt%, respectively, of the weight of NIPAAm, and dissolved with NIPAAm-PEGDA with the aid of an ultrasonic bath for 20 minutes.

A non-thermally-responsive gel is used as the supporting layer for the self-folding bilayer devices. The photo-initiator and solvent are added at 3 wt% and 50 wt% of the weight of PEGDA, respectively, and dissolved by an ultrasonic bath then for 10 minutes. Magnetic nanoparticles (MNPs), 30 nm Fe_3O_4 (PVP coated, Nanostructured and Amorphous), are added at 0.5 vol% of the non-thermally-responsive pregel solution and are dispersed with probe sonicator (4000 mJ, SONICS, USA).

B. Self-folding-hydrogel bilayer fabrication

Self-folding-bilayer structures are fabricated with a two-step backside exposure photolithography processes [14], schematically shown in Fig. 2. A non-thermally-responsive pre-gel solution is first polymerized by UV light (365 nm, 3 mW/cm^2) between the glass mask and a 10 μm thick spacer for 1.5 minutes. Prior to polymerization of the supporting layer, a magnetic field of 10 mT is applied in the planar direction for one minute to align the MNPs in the non-responsive pre-gel solution. The direction of the applied magnetic field determines the folding direction of the structure. Following polymerization of the supporting layer, the spacer is removed and the thermally-responsive-pre-gel solution is introduced into the space between the photomask and the new 30 μm thick spacer substrate. The thermally-responsive layer, which is below the supporting layer, is polymerized by UV-exposure for two minutes. After UV curing, the cell is opened and the bilayers attached to the photomask are released. They fold into tubes once immersed in water.

C. Methods for analyzing the hydrogel nanocomposite

The swelling properties of hydrogel nanocomposites are characterized by measuring their weight at different temperatures in a range from 22˚C to 40˚C. Individual non-thermally-responsive and thermally-responsive layers are polymerized, dried in air, and then hydrated in deionized (DI) water at room temperature. Subsequently, the individual layers are kept in a temperature controlled water bath (Julabo, Germany). The dried (M_s) and swollen (M_d) weights are recorded. The weight swelling ratio (WSR) is defined as

$$\mathrm{WSR} = \frac{M_s - M_d}{M_d} \quad (1)$$

D. Drug release in hydrogel patch

Thermally-responsive-hydrogel disks, with a diameter of 7 mm and thickness of 400 μm, are prepared to analyze the encapsulation and leakage of the drug at both room and body temperature. The hydrogel is polymerized for two minutes between two glass slides that are separated by a stacked cover

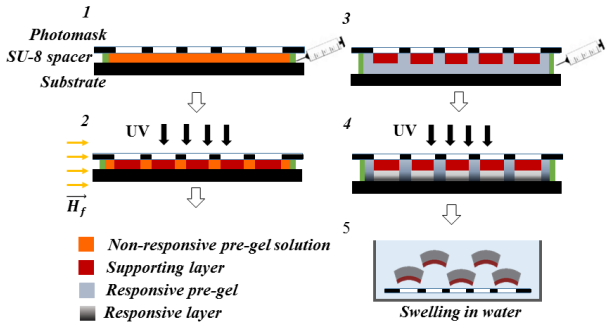

Figure 2. Two-step photolithography process of hydrogel bilayers.

glass. After polymerization, the cell is immersed in DI water for two days. During this period, the hydrogel swells and detaches from the glass slides and any residual un-polymerized pre-gel solution diffuses out from the polymer matrices. A biopsy punch is then used to cut the hydrogel sheet with a defined diameter. The gel disks are then dried in the air for one day and immersed in a model drug solution (Brilliant Green, BG in PBS solution, 1mM) at room temperature for 24 hours. Following this, the disks are removed from the BG solution and immersed in DI water for ten seconds to cleanse residual BG from the surface of gel disks. Then, the gel disks are immersed in 300 μL of PBS solution within an Eppendorf, and the release of the embedded BG from the hydrogel matrices is monitored at room temperature (24˚C) and body temperature (37˚C) for 1 day. Then, all of the samples are monitored at room temperature for ten days. We assume that the total amount of drug is released in this period from the samples immersed at room temperature. At defined periods (every five minutes in the first hour and subsequently every 30 minutes), the PBS solution and any released BG is collected, measured by UV-vis spectroscopy (Infinite M200 Pro, Tecan AG, Mannendorf, Switzerland) at the 622 nm wavelength, and replaced with fresh PBS solution in order to approximate a perfect sink condition.

E. Drug release in hydrogel bilayers at room temperature

The self-folded-hydrogel bilayers are immersed and incubated in a thermostatized water bath at 45˚C for one minute. Since, the temperature is much higher than the lowest-critical-solution temperature (LCST) of NIPAAm (32˚C), the water in the drug-loaded layer will be pressed out. The hydrogel bilayers are then removed and any residual surface water is removed with tissue paper. Subsequently, they are immersed in a 1 mM BG solution at room temperature and are allowed to absorb the model drug for 24 hours. After which, they are immersed in DI water for 10 seconds to rinse off any residual BG. Then, the bilayers are immersed in 300 μL PBS solution within an Eppendorf and the release of the embedded BG is monitored at room temperature for one week. At the same defined periods, the PBS solution and any released BG is collected, measured, and replaced with fresh PBS solution.

F. Drug release in hydrogel bilayers at body temperature

The drug-loading process is the same as above. However, before the hydrogel bilayers are removed from the BG solution, and then heated to 37˚C and sustained for one hour. Afterwards, the bilayers are removed from the BG solution

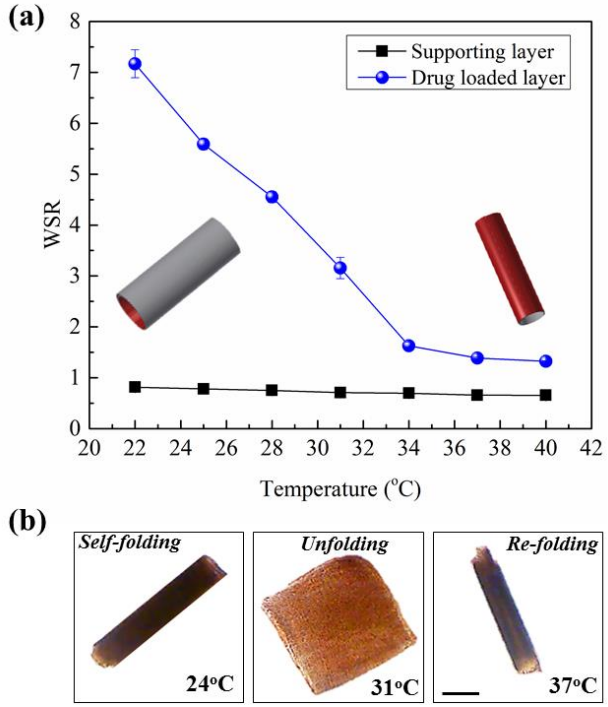

Figure 3. Drug locking mechanism. (a) Thermal responsive weight swelling ratio of individual layers. (b) Optical images of the hydrogel bilayer capsule. The scale bar is 500 μm.

and immersed in DI water at 37˚C to cleanse the residual BG solution for 30 seconds. Subsequently, the bilayers are immersed in 300 μL PBS solution at 37˚C. The release of BG is monitored at 37˚C for two days. The bilayers are then cooled to room temperature and the release is monitored for one week. At the same defined periods, the solution is collected, measured, and replaced with fresh PBS solution at 37˚C.

G. Degradation characterization

To test the degradation rate of individual hydrogel bilayers and supporting layers, both are immersed in sodium hydroxide (NaOH) solution with a concentration of 1 mM, to accelerate the degradation [10], and are kept at 37 ˚C to mimic *in vivo* environments for four days.

III. Results and Discussions

A. Self-folding- and refolding-hydrogel bilayer

A self-folding-hydrogel bilayer is achieved by coupling two material layers with different swelling properties. In this work, a supporting layer with low WSR is coupled with a drug-loading layer with relatively high WSR. The WSR of the supporting layer and drug-loaded layer at various temperatures are shown in Fig. 3a. The resulting shape of the hydrogel bilayers is reconfigurable because the NIPAAm hydrogel is thermally responsive. As the temperature increases, the WSR of the drug-loaded layer decreases and triggers an unfolding process. When the temperature is higher than the LCST, the hydrogel bilayer refolds in the opposite sense, and the WSR of the two layers becomes constant. When the ambient temperature is much lower than the LCST, the bilayer folds into a cylindrical tube with the drug-loaded layer exposed to the external environment. While the temperature is higher than the LCST, the bilayer refolds in the opposite sense

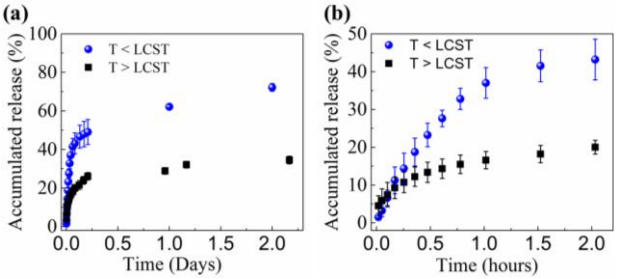

Figure 4. Drug releasing profile in NIPAAm gel patch measured at the temperature higher and lower than the LCST. (a) Releaseover two days. (b) Release within two hours.

and the drug-loaded layer is hidden on the inner side of the cylindrical tube. Figure 3b shows optical images of the hydrogel bilayer at three different temperature. Starting from 24˚C, the bilayer completely unfolds by 31˚C and then refolds into a compact tube by 37˚C.

B. Thermally-responsive hydrogel drug release

The amount of the drug solution loaded into the hydrogel is determined by the WSR, which is proportional to the pore size. Larger pore size allows the hydrogel to load more drug but results in a higher drug-leakage rate. By increasing the temperature to body temperature, the hydrogel matrix collapses (reducing the pore size), the diffusion rate decreases, and the drug is encapsulated. After two days, the hydrogel releases 70% of the loaded drug if left at room temperature (T< LCST). However, within the same duration of time, the hydrogel releases only 30% of the loaded drug at body temperature (Fig. 4a). One of the main challenges in certain targeted drug delivery systems such as HRMDs is to avoid the initial burst release. Fig. 4b shows the accumulated release of the drug within two hours. The drug leakage at high temperature is less than 20%, whereas the drug leakage at room temperature approaches 50%. The initial release at high temperature, shown in Fig. 4b, is slightly higher than the release at low temperature, because of the squeezing effect of the hydrogel during the transition from room temperature to body temperature. Although nearly 5% of the loaded drug is lost during encapsulation, the reduced diffusion rapidly offsets the initial losses.

C. Drug release in self-folded and re-folded bilayers

The use of a thermally-responsive hydrogel for the encapsulation of a drug can be improved by isolating the drug layer from the environment. Refolding transforms the drug layer from the outer to inner layer. Moreover, an additional supporting layer can further reduce the drug diffusion rate. Figure 5 shows the leakage of drugs from the self-folded tubes at room temperature and the refolded tubes at body temperature. In order to prevent the squeezing effects observed in Fig. 4b, the refolded tubes are incubated in the BG solution at 37˚C and transferred to a PBS solution at the same temperature. Within three hours, 80% of the BG is released by the self-folded tubes at room temperature. However, within the same time period, the refolded tubes release only 10% of the BG. The primary leakage path in the refolded tubes is from the tube ends. In this configuration, the

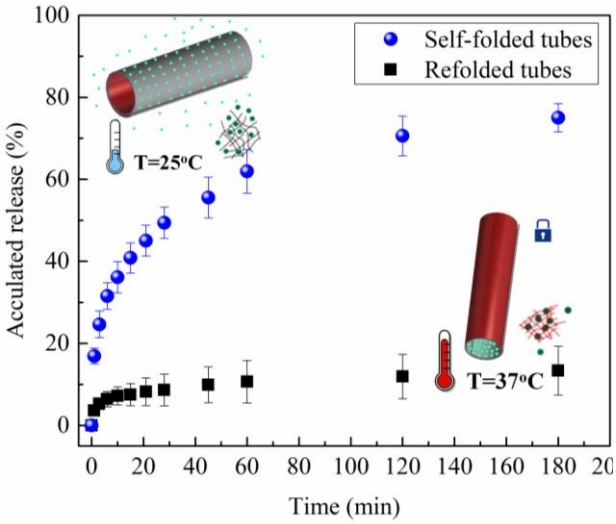

Figure 5. Drug release rate of the self-folded bilayers and the refolded bilayers. The supporting layer shields the loaded drug from the environment at body temperature, which further reduces the diffusion rate compared to room temperature.

tubes could be used as controlled directional release devices for long durations.

Furthermore, the bilayer tube can be manipulated with magnetic manipulation system because of the embedded magnetic nanoparticles in the supporting layer. Figure 6 shows magnetic manipulation of the refolded tube controlled by a magnetic manipulation system called the Octomag [15].

D. Degradation of the supporting layer

Both refolded-bilayer tubes and individual supporting layers are immersed in a NaOH solution at 37˚C. It was observed that the individual supporting layers completely degraded and the bilayer tubes unfolded within one hour. Degradation of the remaining drug-loaded layer requires four days to complete.

CONCLUSION

This work proposes a self-folding-hydrogel bilayer with the ability to encapsulate drug and minimize its leakage to the environment by refolding its shape. Although the device has a 5% reduction in drug payload due to the encapsulation, the experimental results show a significant reduction in drug leakage compared to conventional self-folding-hydrogel bilayers. The proposed bilayer tube shows great potential for the use in *in vivo* targeted drug delivery applications.

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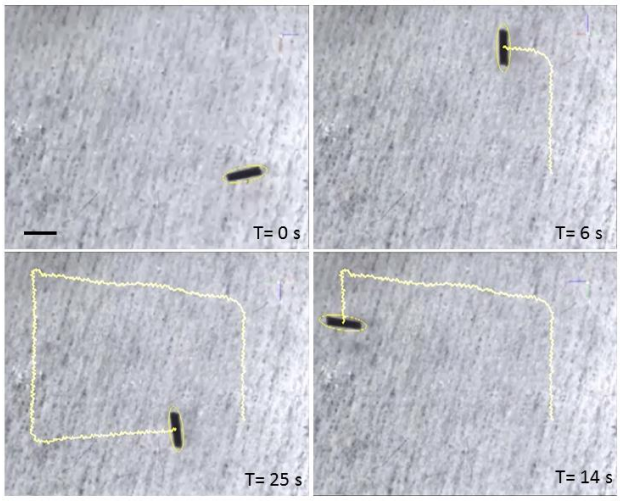

Figure 6. Magnetic manipulation of the mobile refolded capsule. The yellow line shows the trajectory recorded by image process. The scale bar is 1 mm.

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